

6.—Copper, Molybdenum and Inorganic Sulphate Levels in Rottnest Plants

By S. Barker*

Manuscript received—16th February, 1960

Determinations of the copper, molybdenum and inorganic sulphate levels of plants from Rottnest Island, Western Australia, are reported. These analyses were undertaken during an investigation to determine whether the quokka, *Setonix brachyurus* (Quoy & Gaimard) population of the island suffers a seasonal copper deficiency.

Introduction

Shield (1959, p. 78) suggests that semi-starvation producing "a rather profound anaemia of the order of 25 per cent. decrease in the haemoglobin, red cell count, and haematocrit" is one of the principal causes of death in the Rottnest quokka, *Setonix brachyurus* (Quoy & Gaimard). Moir, Somers, Sharman and Waring (1954) and Moir, Somers and Waring (1956) have shown that the quokka has ruminant-like digestion, and this suggests that it, like the true ruminant, may show anaemia due to dietary deficiencies of "trace elements" such as cobalt and copper.

It has been shown in the sheep that: ingestion of inorganic sulphate increases the urinary excretion of molybdenum with a corresponding fall in blood molybdenum levels (Dick 1953a); high molybdenum and inorganic sulphate intakes for long periods exert a depressing effect on liver copper storage (Dick 1953b); experimental hypocuprosis can be produced by feeding intake levels of copper, molybdenum and inorganic sulphate similar to those occurring in pastures where grazing sheep develop hypocuprosis (Wynne and McClymont 1955).

Barker (1960) has shown that inorganic sulphate influences excretion of molybdenum in the urine, and blood molybdenum levels in the quokka. It has also been found in the quokka that the long term ingestion of raised intake levels of inorganic sulphate and molybdenum depresses liver copper storage (Barker, unpublished data). Thus it is possible that anaemia reported by Shield (1959) may be due in part to either direct or induced hypocuprosis.

The work required to test the thesis that copper deficiency causes a seasonal anaemia in the Rottnest quokka population is divided into four parts which are listed below:—

- (1) Determination of the copper, molybdenum and inorganic sulphate levels of food plants on Rottnest.
- (2) Quantitative determination of the various food plants constituting the diet of the quokka through the seasons.

From these data copper, molybdenum and inorganic sulphate intakes can be calculated.

- (3) Estimation of haematological values and blood copper and molybdenum levels throughout the seasons of the year.
- (4) Diagnoses of copper deficiency and the copper, molybdenum and inorganic sulphate levels at which an induced deficiency occurs.

The present paper gives the results of part (1) above. Part (2) is covered by work being carried out by G. M. Storr (Zoology Department, University of Western Australia) and will be reported elsewhere. The results obtained by the present author under parts (3) and (4) will be published later.

Methods

Determination of the precise composition of the diet (by plant species) is only possible by the methods of Storr [(2) above]. However, simple observation is sufficient to show that some plants are only eaten at certain seasons of the year while others are eaten over a more prolonged period. Furthermore a few plants which are heavily and persistently grazed, e.g. *Sporobolus*, are so restricted in their occurrence that it is inconceivable that they constitute a significant proportion of the diet of a large part of the population. These considerations were taken into account when plant samples were collected on ten occasions between December, 1957, and May, 1959, and only those portions of species actually being eaten at the time were collected.

Plants were collected by hand and placed in individual sample bags, and the bags were then tied inside a polythene sack. They were transported to the mainland laboratory on the afternoon of collection and were processed immediately. Gravimetric moisture determinations were made by weighing before and after heating in an oven at 100°C. for 24 hours. Dried samples were crushed in an all steel mill. For copper and molybdenum estimation, samples were wet digested in nitric, sulphuric and perchloric acids. Copper was estimated by the method of Eden and Green (1940) and molybdenum by the method of Piper and Beckworth (1948). Inorganic sulphate was estimated by the method of Dick and Bingley, the endpoint was determined by titration (Dick 1954).

Results

The mean results of copper, molybdenum and inorganic sulphate analyses and moisture determinations of plants collected at the West End

* Zoology Department, Washington State University, Pullman, Washington, U.S.A. Formerly Zoology Department, University of Western Australia, Nedlands, Western Australia.

and Lake Bagdad study areas between December, 1957, and June, 1959, are presented in Tables I and II.

The results of individual analyses carried out on two plant species are presented in Table III to show seasonal variation. The common "pigface" (*Carpobrotus*) is found in limited areas at West End and the "saltwater couch" (*Sporobolus*) on the edge of Lake Bagdad and at other lakes. Both plants are eaten extensively by the quokka.

Discussion

Bennetts and Beck (1942) listed copper levels of plants growing on copper deficient soils at Gingin. They considered that sheep would become deficient when grazing pastures containing less than 3.0 p.p.m. copper on a dry weight basis. They classified pastures containing 3.0 p.p.m. copper or less as deficient, 3.0 to 6.0 p.p.m. as marginal and more than 6.0 p.p.m. as normal. All levels were expressed on a dry weight basis. Using the classification of Bennetts and Beck, it is herein considered that on a sheep-basis

Rottnest plants are copper deficient. The only species analysed more than once that had consistently normal copper levels was *Eremophila glabra*. It seems likely that this species is a copper accumulator.

Plant molybdenum levels were low in both areas, but Lake Bagdad plant levels were higher than West End plant levels. There are no figures available for comparison from Western Australian mainland plants.

There are no published figures of inorganic sulphate levels in Australian plants. However, Beck (personal communication) has found that sheep pastures in Western Australia normally have inorganic sulphate levels ranging from 0.2 to 0.3%. In the present study mean inorganic sulphate levels in plants collected in the study areas were as high as 2% in some species. On a sheep-basis this level is high.

It can be seen from Table III that there is little change in copper, molybdenum and inorganic sulphate levels of *Carpobrotus* at different times of the year. The increase of copper and molybdenum levels in *Sporobolus* during

TABLE I

Copper, molybdenum and inorganic sulphate levels of plants collected from the Lake Bagdad area, Rottnest, between December, 1957, and May, 1959, expressed on a dry weight basis.

Species	No. of samples	Moisture %	Cu p.p.m.	Mo p.p.m.	SO ₄ ²⁻ %
<i>Arthrocnemum halocnemoides</i>	10	83 (80-90)	2.5 (1.1-5.1)	0.6 (0.4-1.0)	1.6 (0.9-2.3)
<i>Carex preissii</i>	1	76	2.7	5.2	1.4
<i>Ehrharta longiflora</i>	3	81 (69-88)	3.8 (2.4-4.9)	5.4 (3.9-6.1)	0.2 (0.1-0.2)
<i>Erythraea centaurium</i>	1	79	8.0	2.6	0.4
<i>Euphorbia peplus</i>	1	85	2.4	3.6	0.5
<i>Gahnia trifida</i> (leaves)	8	35 (22-42)	1.4 (0.6-1.9)	0.6 (0.4-0.9)	0.2 (0.2-0.3)
<i>Gahnia trifida</i> (seed head)	1	48	2.4	0.3	0.1
<i>Melaleuca pubescens</i>	3	59 (56-61)	2.4 (2.1-2.8)	0.6 (0.5-0.8)	0.81 (0.6-0.9)
<i>Rhagodia baccata</i>	2	78 (74-83)	1.5 (1.1-1.9)	0.6 (0.4-0.9)	0.5 (0.5)
<i>Salicornia australis</i>	10	86 (79-90)	2.3 (0.7-4.8)	0.4 (0.2-0.7)	0.9 (0.7-1.3)
<i>Samolus repens</i>	3	62 (50-75)	5.9 (5.8-6.0)	3.4 (1.6-4.5)	2.0 (1.8-2.3)
<i>Sonchus oleraceus</i>	1	92	3.6	3.3	0.6
<i>Solanum simile</i> (berries)	5	70 (69-72)	1.7 (1.4-2.0)	0.9 (0.7-1.3)	0.2 (0.1-0.3)
<i>Solanum simile</i> (leaves)	1	81	2.3	3.6	1.0
<i>Sporobolus virginicus</i>	10	58 (50-75)	3.9 (2.7-6.4)	5.4 (1.9-16.6)	1.0 (0.3-2.0)
<i>Stipa variabilis</i>	2	48 (37-60)	1.8 (1.3-2.4)	3.1 (3.1-3.2)	0.3 (0.3-0.4)
<i>Templetonia retusa</i>	1	57	1.1	0.1	0.2
<i>Threlkeldia diffusa</i>	3	88 (83-92)	2.8 (2.3-3.1)	0.6 (0.5-0.8)	0.5 (0.3-0.8)
<i>Vulpia myuros</i>	3	76 (69-82)	4.4 (3.3-6.6)	3.5 (1.7-4.7)	0.3 (0.2-0.5)

TABLE II

Copper, molybdenum and inorganic sulphate levels of plants collected from the West End area, Rottneest, between December, 1957, and May, 1959, expressed on a dry weight basis.

Species	No. of samples	Moisture %	Cu p.p.m.	Mo p.p.m.	SO ₄ ²⁻ %
<i>Acacia rostellifera</i> (bark)	4	49 (39-57)	1.7 (1.2-2.2)	0.9 (0.5-1.2)	1.7 (1.5-1.8)
<i>Acacia rostellifera</i> (leaves)	4	69 (66-77)	1.4 (1.0-1.9)	0.5 (0.1-1.0)	1.0 (0.8-1.3)
<i>Atriplex cinerea</i>	2	79 (72-86)	1.8 (1.2-2.4)	0.5 (0.3-0.7)	1.0 (0.8-1.3)
<i>Bromus gussonii</i>	1	87	9.7	0.3	0.4
<i>Carpobrotus aequilaterus</i>	10	90 (87-94)	0.8 (0.4-1.9)	0.1 (0.0-0.2)	0.4 (0.2-0.6)
<i>Enchylaena tomentosa</i>	2	82 (75-90)	4.1 (4.0-4.1)	0.4 (0.3-0.5)	1.2 (0.9-1.4)
<i>Eremophila glabra</i>	4	73 (66-80)	7.8 (4.7-11.5)	0.5 (0.1-1.1)	0.7 (0.5-0.9)
<i>Euphorbia peplus</i>	1	93	3.8	1.2	0.5
<i>Frankenia pauciflora</i>	4	41 (34-56)	2.3 (1.1-2.9)	0.3 (0.3-0.4)	1.8 (1.4-2.0)
<i>Melilotus indica</i>	1	80	5.0	0.1	0.4
<i>Nitraria schoberi</i>	2	84 (79-89)	1.2 (0.7-1.7)	0.2 (0.1-0.3)	0.6 (0.4-0.7)
<i>Olearia axillaris</i>	1	58	2.1	0.5	0.2
<i>Poa australis</i>	1	61	2.4	1.8	0.4
<i>Rhagodia laccata</i>	9	82 (75-89)	1.3 (0.5-2.7)	0.4 (0.1-0.5)	0.8 (0.5-1.2)
<i>Scaevola crassifolia</i> (bark)	3	44 (42-47)	3.6 (2.5-4.9)	0.2 (0.1-0.3)	1.3 (0.9-1.8)
<i>Scaevola crassifolia</i> (leaves)	7	75 (71-83)	2.8 (1.9-3.7)	0.5 (0.3-0.6)	1.4 (1.1-1.8)
<i>Solanum simile</i> (berries)	1	72	1.4	0.8	0.2
<i>Solanum simile</i> (leaves)	1	78	1.6	2.3	1.6
<i>Stipa variabilis</i>	4	46 (31-64)	1.7 (1.1-2.2)	0.7 (0.6-0.7)	0.3 (0.2-0.6)
<i>Tetragonia implexicoma</i>	7	85 (74-92)	1.9 (0.9-2.9)	0.1 (0.0-0.2)	2.2 (0.4-3.8)
<i>Threlkeldia diffusa</i>	3	81 (78-85)	1.3 (1.1-1.5)	0.3 (0.3-0.4)	0.6 (0.5-0.7)
<i>Westringia dampieri</i>	1	49	1.1	9.1	0.1

the summer months is possibly caused by faecal contamination from the increased number of animals which graze on the plant at this time of the year.

It will not be possible to calculate seasonal changes in trace element intake levels of quokkas on Rottneest until intake of the various plant species has been estimated quantitatively. However, the results of field and laboratory experiments show that quokkas in the Lake Bagdad area exhibit a seasonal anaemia associated with copper depletion, those living at West End do not (Barker, unpublished data).

Acknowledgments

This work was made possible by annual grants from the University of Western Australia Research Grants Committee and assistance from

the Commonwealth Scientific and Industrial Research Organisation. The writer and a full-time assistant, Mr. C. J. Geeves, were in receipt of salary paid from a Rockefeller Foundation Research Grant to Professor H. Waring. Mr. A. B. Beck, C.S.I.R.O. Division of Animal Health and Production is thanked for technical advice. This work was part of a Marsupial Research Project under the general direction of Dr. A. R. Main to whom the writer is deeply indebted for assistance.

References

- Barker, S. (1960).—Effect of inorganic sulphate on the excretion of molybdenum by a marsupial. *Nature, Lond.* 185: 41-42.
- Bennetts, H. W., and Beck, A. B. (1942).—Enzootic ataxia and copper deficiency of sheep in Western Australia. *Bull. Coun. Sci. Indust. Res. Aust.* 147.

- Dick, A. T. (1953a).—The effect of inorganic sulphate on the excretion of molybdenum in the sheep. *Aust. Vet. J.* 29: 18-26.
- Dick, A. T. (1953b).—Influence of inorganic sulphate on the copper-molybdenum interrelationship in sheep. *Nature, Lond.* 172: 637-638.
- Dick, A. T. (1954).—Studies in the investigation of toxæmic jaundice of sheep. D.Sc. Thesis. Univ. Melbourne, Vict.
- Eden, A., and Green, H. H. (1940).—Micro-determination of copper in biological material. *Biochem. J.* 34: 1202-1208.
- Moir, R. J., Somers, M., Sharman, G. B., and Waring, H. (1954).—Ruminant-like digestion in a marsupial. *Nature, Lond.* 173: 269-270.
- Moir, R. J., Somers, M., and Waring, H. (1956).—Studies on marsupial nutrition. 1. Ruminant-like digestion in a herbivorous marsupial (*Setonix brachyurus* Quoy & Gaimard). *Aust. J. Biol. Sci.* 9: 293-304.
- Piper, C. S., and Beckworth, R. S. (1948).—A new method for determination of small amounts of molybdenum in plants. *J. Soc. Chem. Ind. Lond.* 67: 374-378.
- Shield, J. W. (1959).—Rottnest field studies connected with the quokka. *J. Roy. Soc. W. Aust.* 42: 76-78.
- Wynne, K. N., and McClymont, G. L. (1955).—Copper-molybdenum-sulphate interaction in induction of hypocuprosis. *Nature, Lond.* 175: 471-472.

TABLE III

Seasonal changes in the moisture, copper, molybdenum, and inorganic sulphate levels of a grass (*Sporobolus*) collected at Lake Bagdad, and a succulent (*Carpobrotus*) collected at West End.

DATE COLLECTED	<i>Carpobrotus aequilaterus</i>				<i>Sporobolus virginicus</i>			
	Moisture %	Cu p.p.m.	Mo p.p.m.	SO ₄ %	Moisture %	Cu p.p.m.	Mo p.p.m.	SO ₄ %
23/12/57	90	0.9	0.2	0.5	53	2.7	3.9	2.0
18/1/58	88	0.7	0.0	0.5	57	3.3	4.6	1.7
25/2/58	89	0.6	0.1	0.5	57	6.0	8.1	1.3
14/4/58	86	0.8	0.1	0.6	51	3.3	5.0	0.8
26/5/58	89	0.6	0.0	0.4	50	3.5	3.4	0.6
22/6/58	94	0.8	0.1	0.2	60	4.4	2.9	0.4
5/8/58	93	0.8	0.2	0.2
22/10/58	92	1.9	0.2	0.4	75	6.4	1.9	0.7
5/12/58	89	0.7	0.2	0.4	53	3.0	16.6	1.5
23/2/59	87	0.4	0.2	0.2	62	2.7	5.2	0.3
3/6/59	62	4.2	2.8	0.4